

FIGURE 1. Pro104.D116.1 MAb Binds to 293F Cells Transiently Transfected with Pro104

Fig. 1A

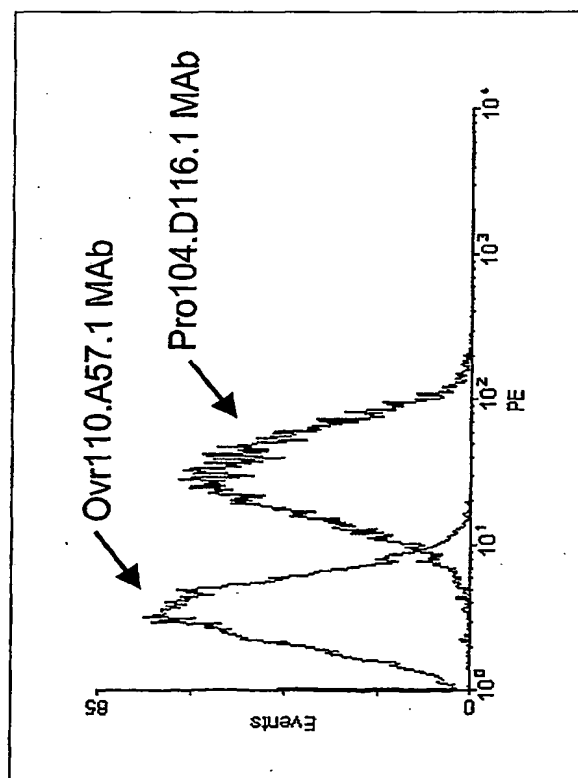
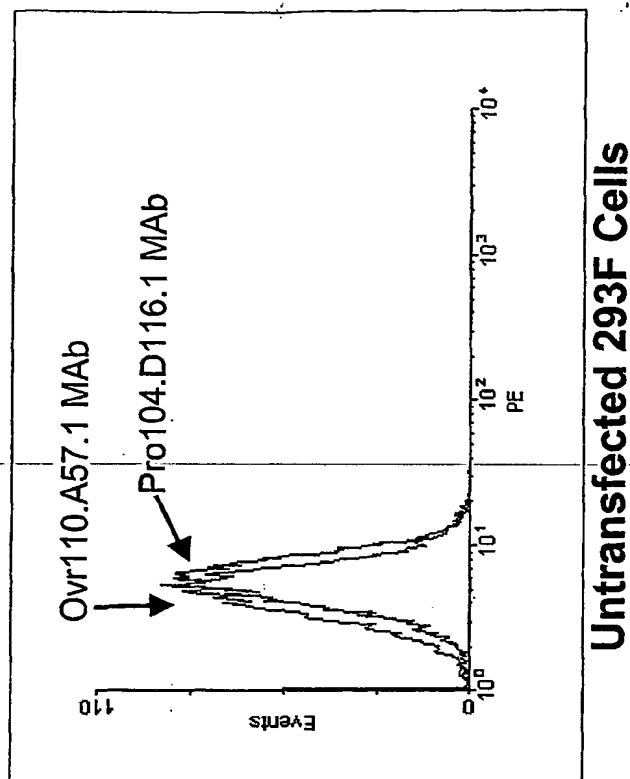


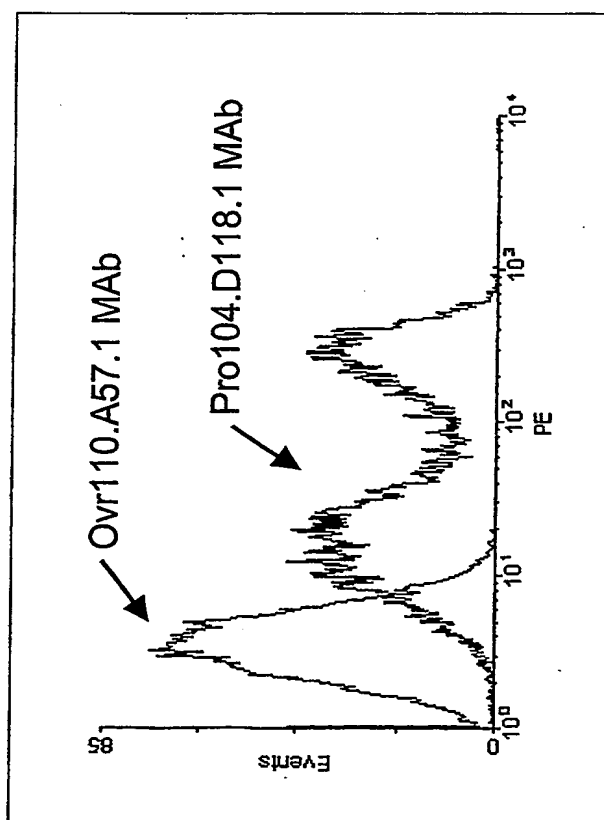
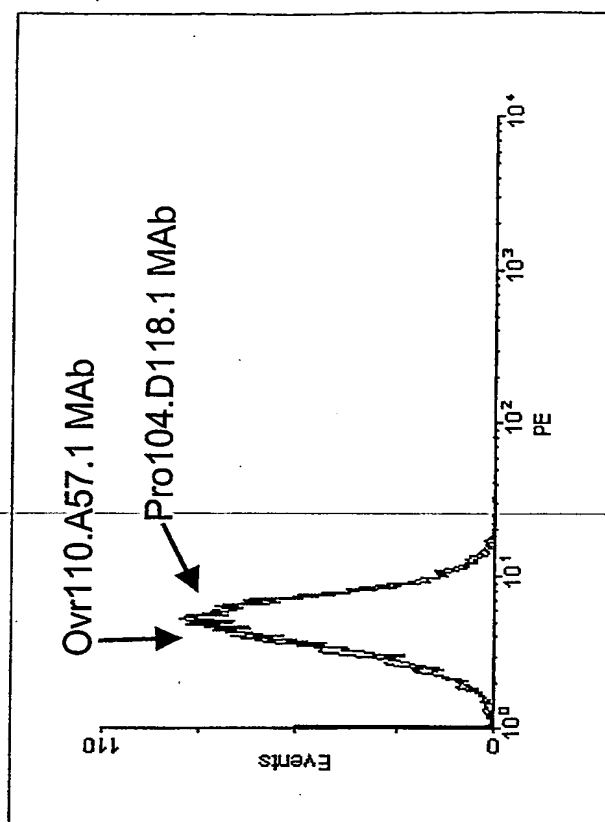
Fig. 1B



No Fill: Pro104.D116.1 MAb
Shaded: Ovr110.A57.1 Control MAb

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FIGURE 2. Pro104.D118.1 MAb Binds to 293F Cells Transiently Transfected with Pro104

Fig. 2A**Pro104-Transfected 293F Cells****Fig. 2B****Untransfected 293F Cells**

No Fill: Pro104.D118.1 MAb
Shaded: Ovr110.A57.1 Control MAb

**FIGURE 3. Pro104.C19.1 binds to live HeLa
Cancer Cells Expressing Pro104**

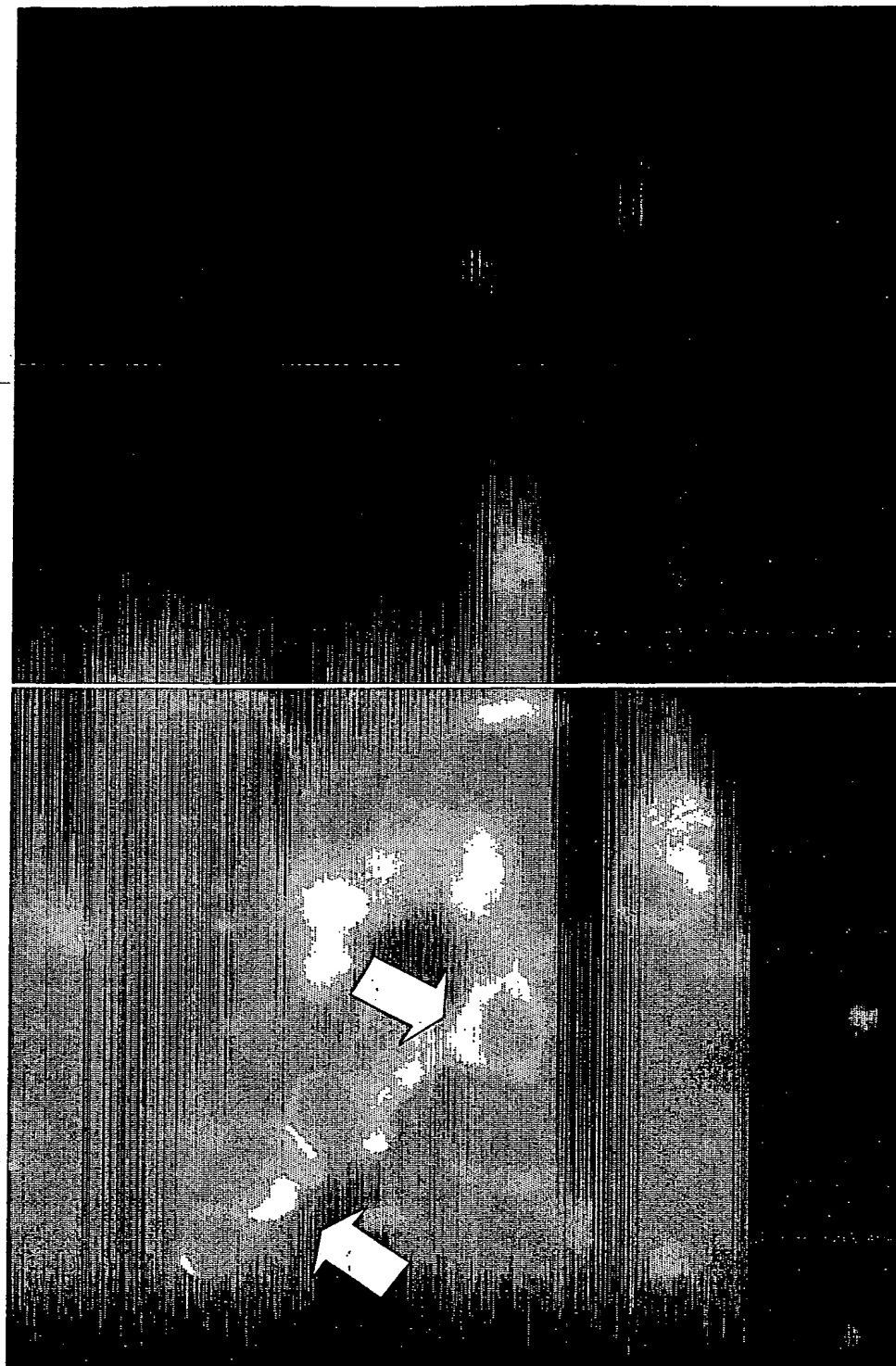


Fig. 3A. HeLa Cells

Fig. 3B. SKOV-3 cells

N= Nuclei

**FIGURE 4. Cy3-Pro104.C25.1 binds to live HeLa
Cancer Cells Expressing Pro104**

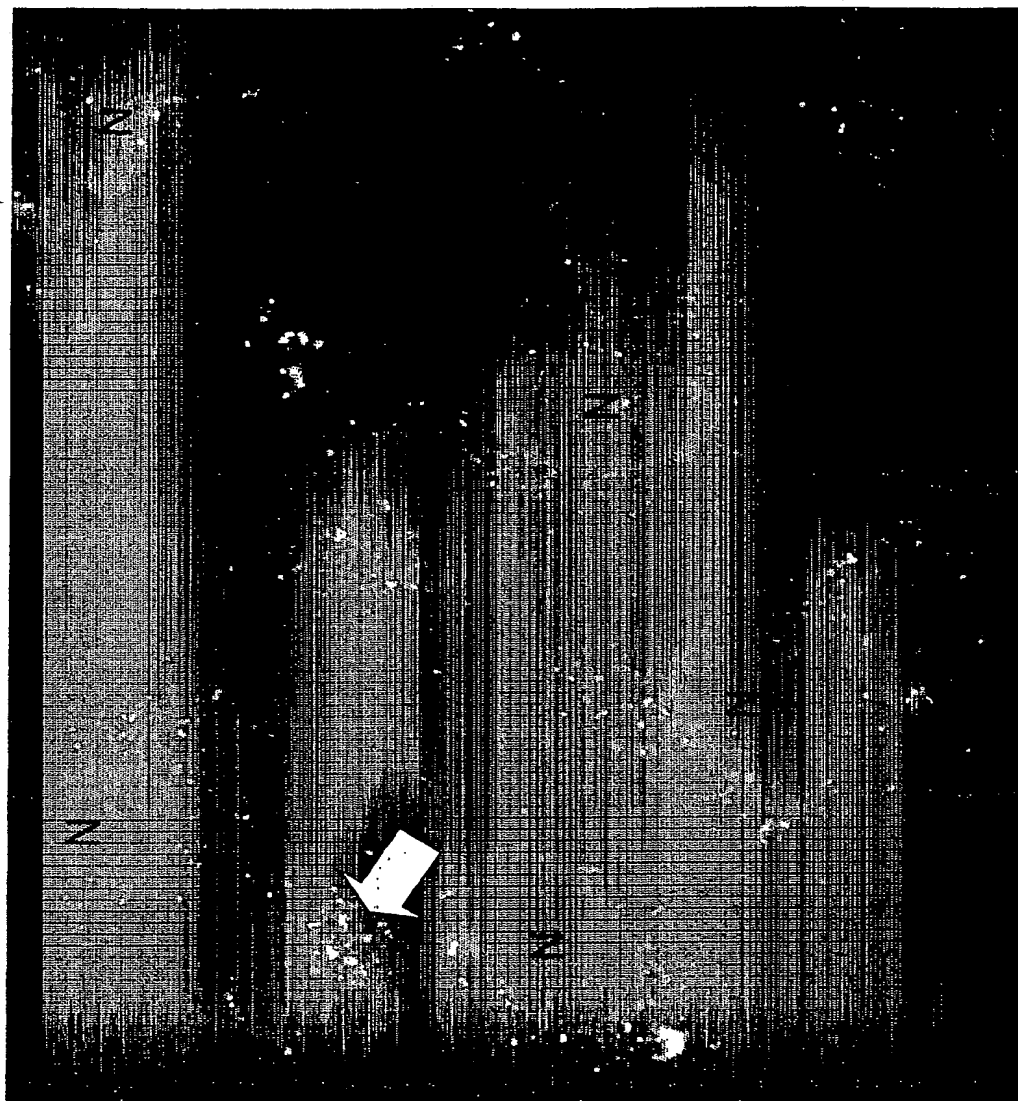


Fig. 4A. HeLa Cells

Fig. 4B. SKOV-3 cells

N= Nuclei

**FIGURE 5. Cy3-Pro104.C25.1 binds to and is Internalized
in live HeLa Cancer Cells Expressing Pro104**



N= Nuclei

**FIGURE 6. Cy3-Pro104.C19.1 binds to and is Internalized
in Pancreatic Cancer Cells Expressing Pro104**

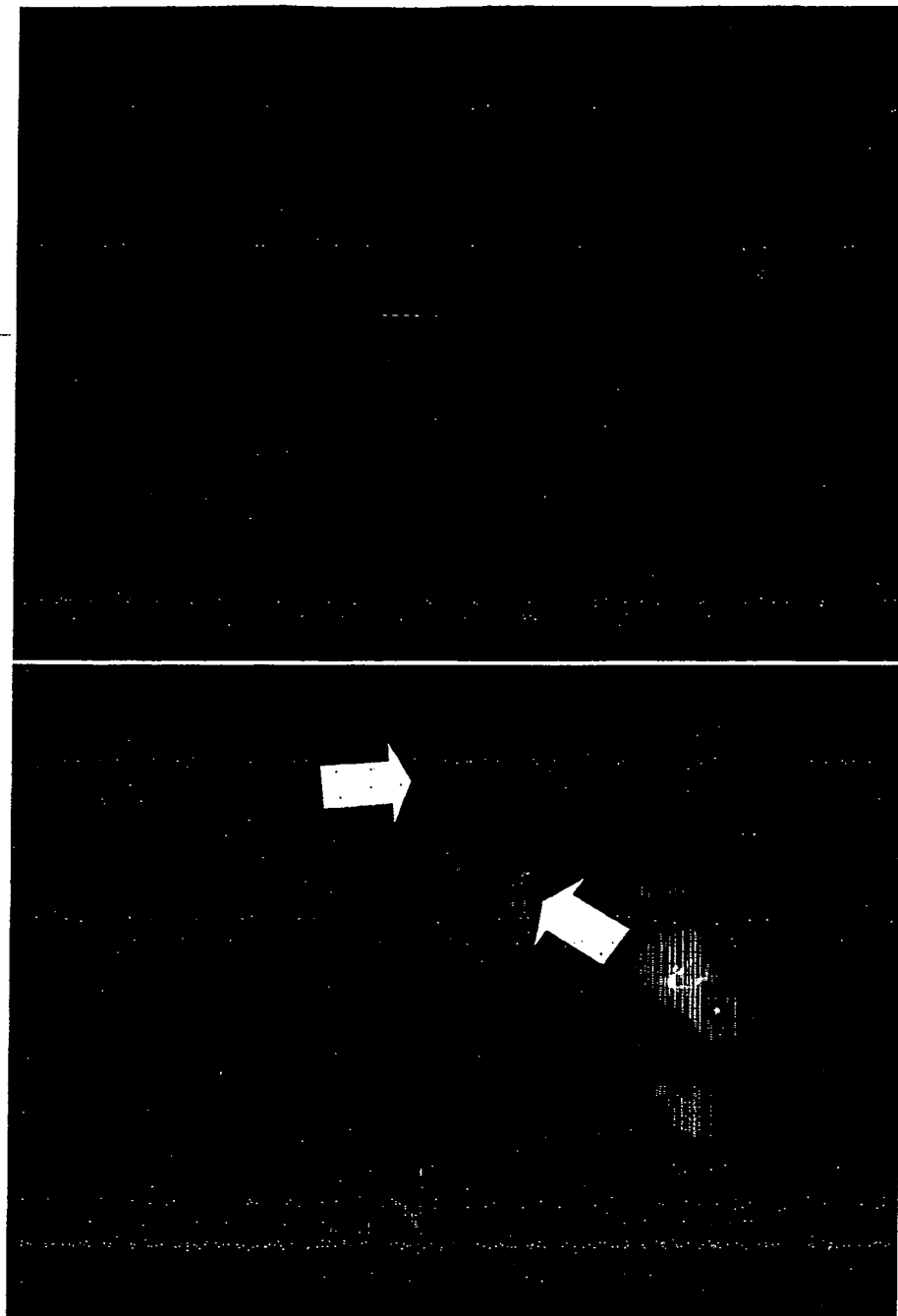


Fig. 6A. MIA PaCa-2 Cells

Fig. 6B. HCT-116 cells

N= Nuclei

**FIGURE 7. Cy3-Pro104.C55.1 binds to and is Internalized
in Pancreatic Cancer Cells Expressing Pro104**

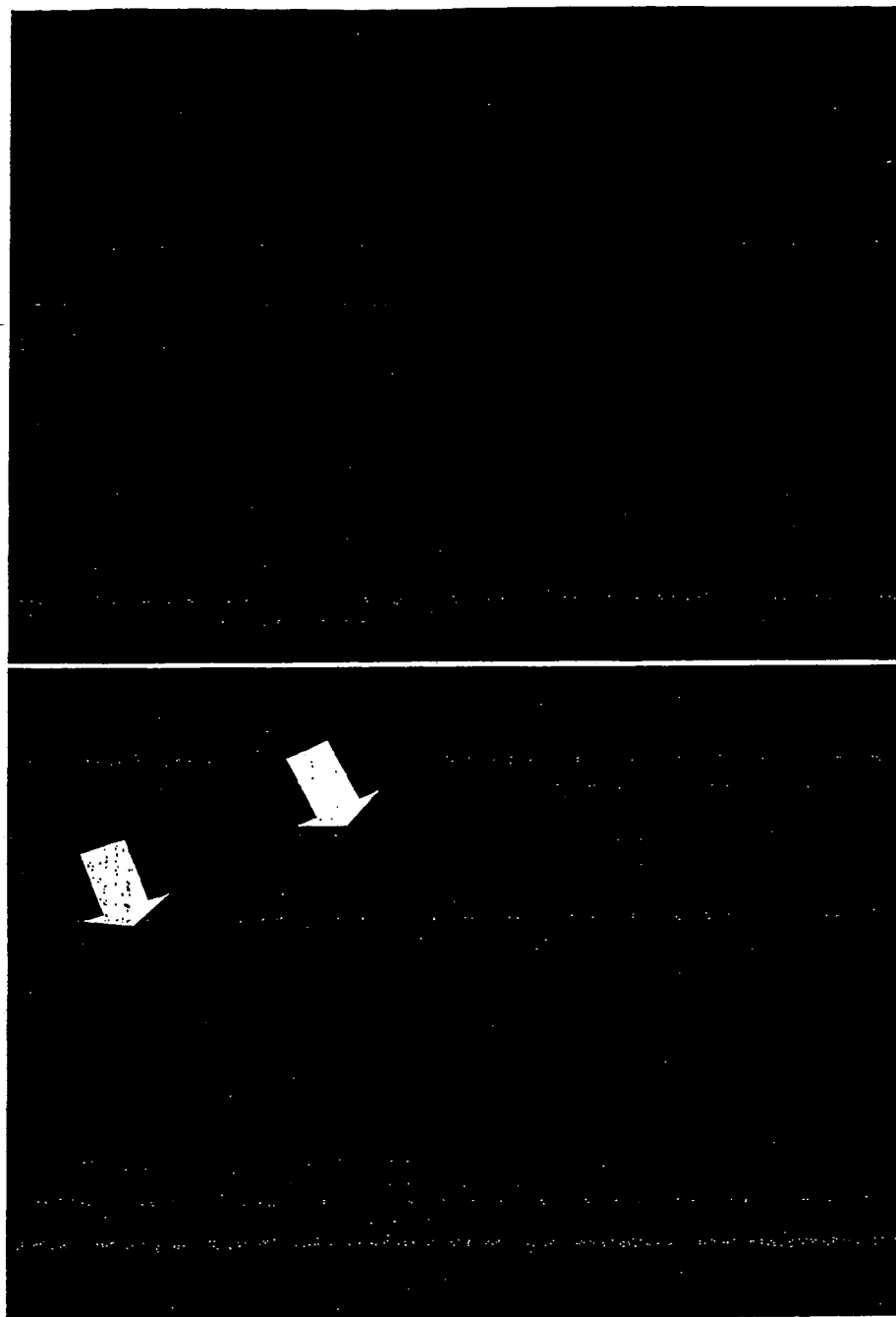


Fig. 7A. MIA PaCa-2 Cells

Fig. 7B. HCT-116 cells

N= Nuclei

**FIGURE 8. Pro104.C25.1 binds to Pro104 on
Cancer Cells in Ovarian Tumors**

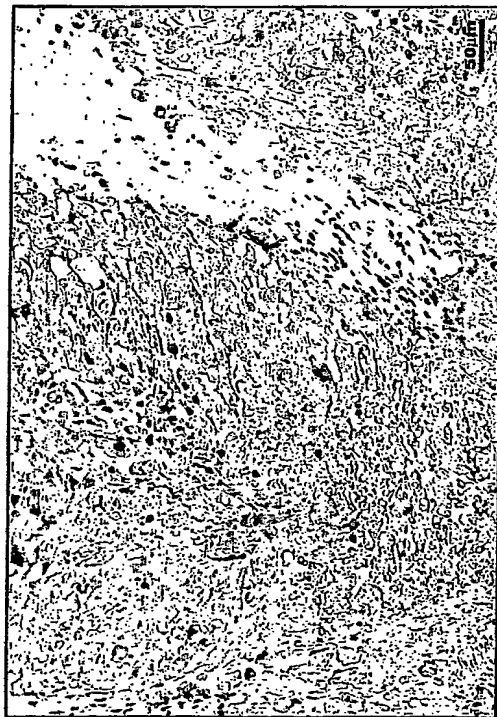


Fig. 8A ovarian cancer 1



Fig. 8B normal ovary 1



Fig. 8C ovarian cancer 2

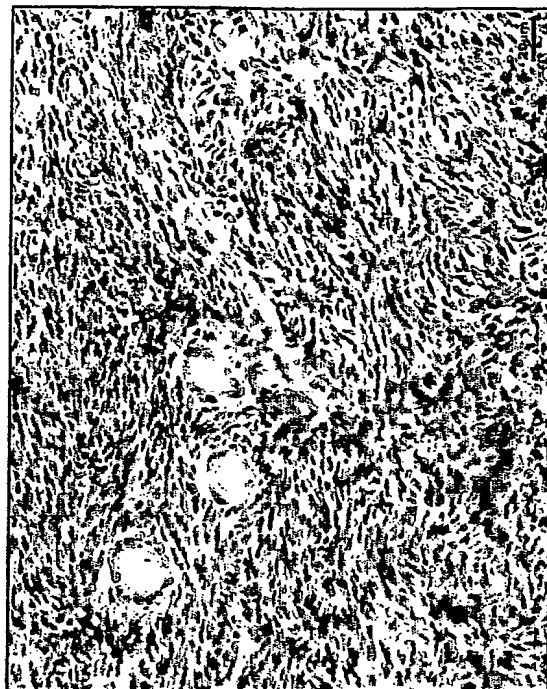
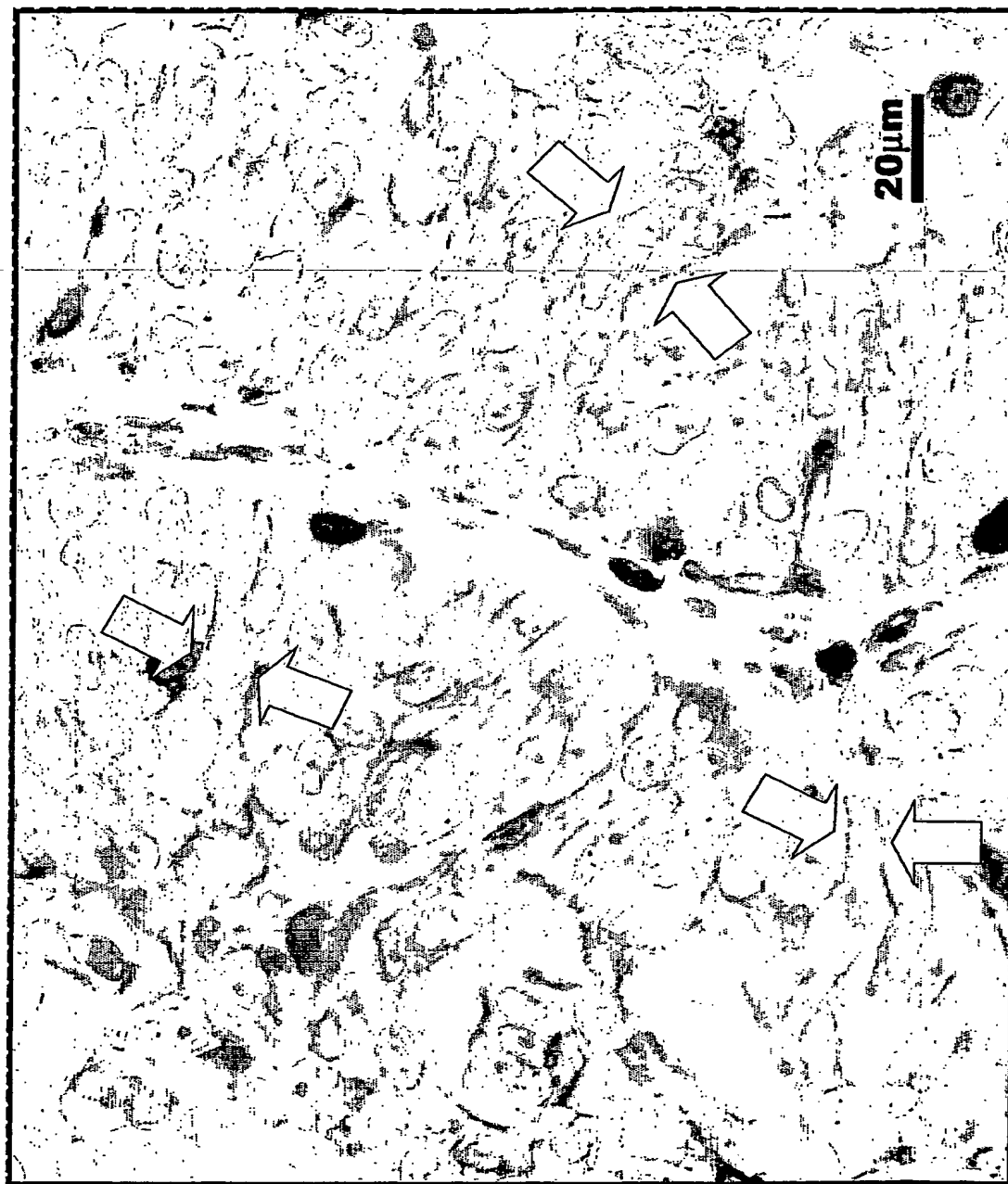


Fig. 8D normal ovary 2

**FIGURE 9. Pro104.C25.1 binds to Pro104 on the Cell
Membrane of Ovarian Cancer Cells**



**FIGURE 10. Pro104.D9 binds to Pro104 on the Cell
Membrane of Ovarian Cancer Cells**



**FIGURE 11. Pro104.D133 binds to Pro104 on the Cell
Membrane of Serous Ovarian Cancer Cells**

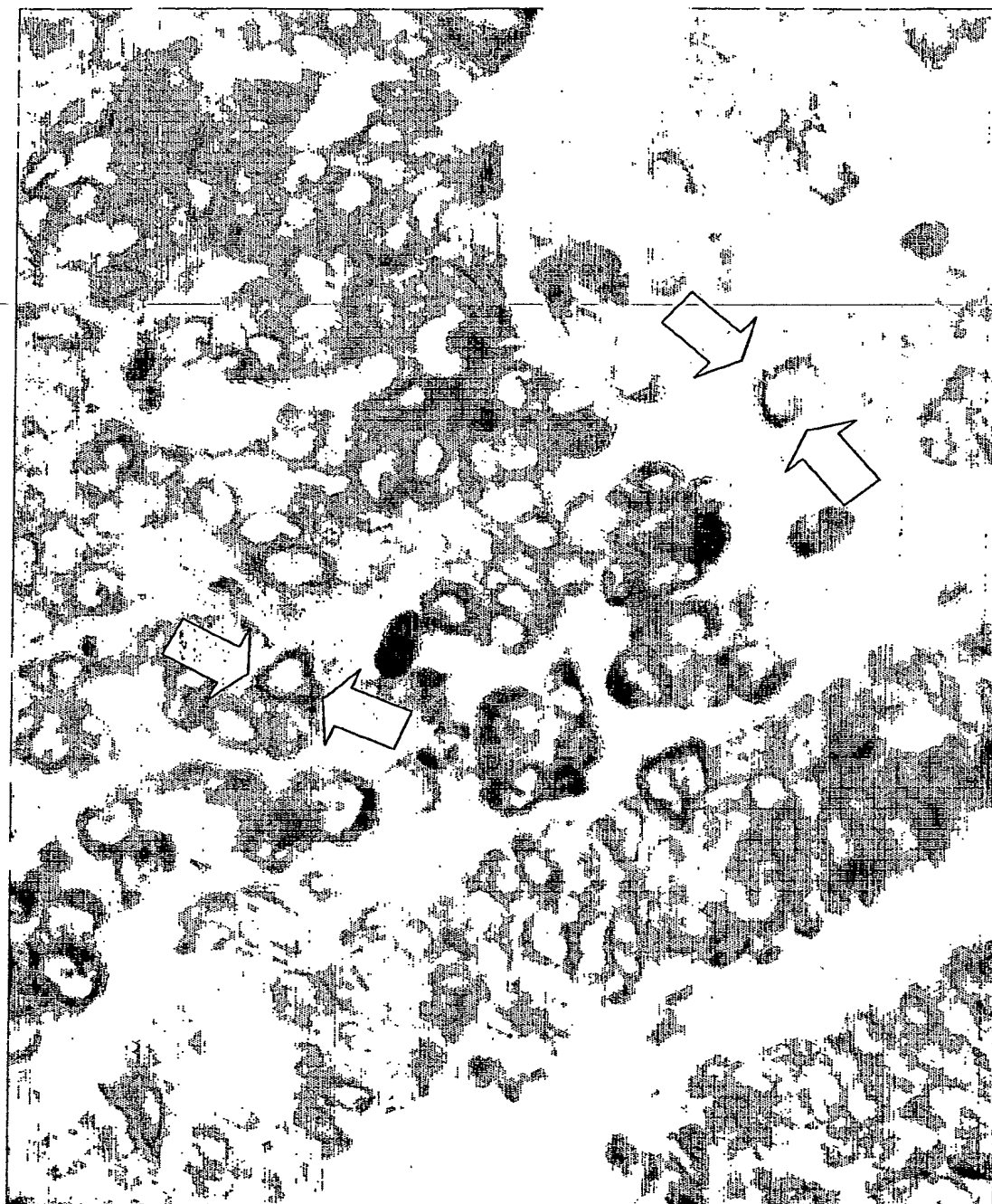


FIGURE 12. Pro104.C25.1 binds to Pro104 on Cancer Cells in Pancreatic Tumors



Fig. 12A Pancreatic cancer 1

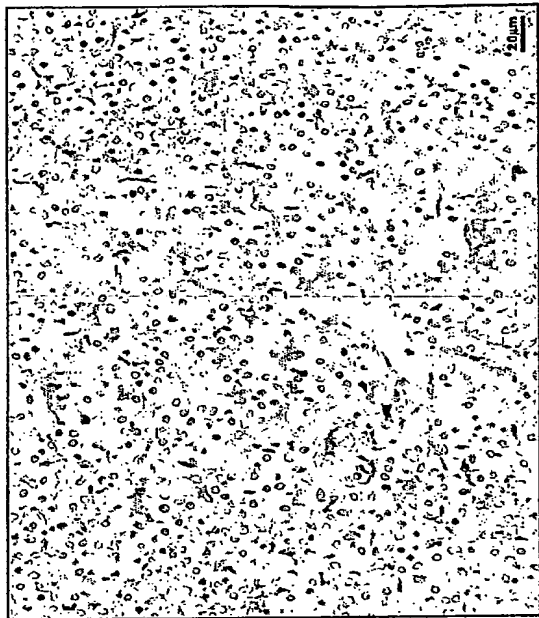


Fig. 12B Normal pancreas 1



Fig. 12C Pancreatic cancer 2

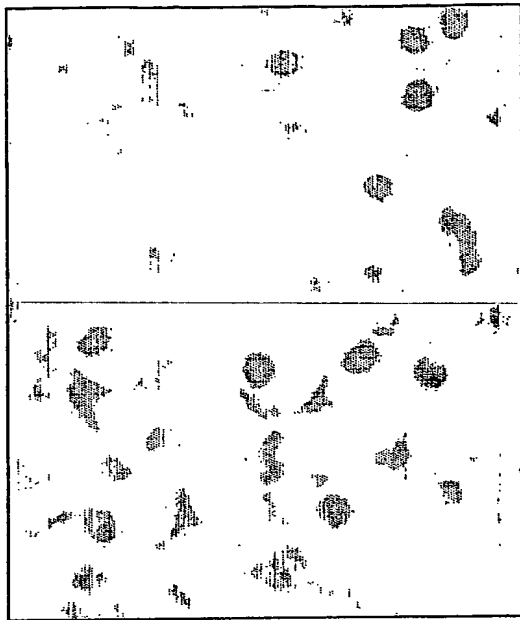


Fig. 12D Normal pancreas 2

FIGURE 13. Controls Demonstrating Pro104 MAb Immunolabeling Specificity



Fig. 13A Mouse IgG

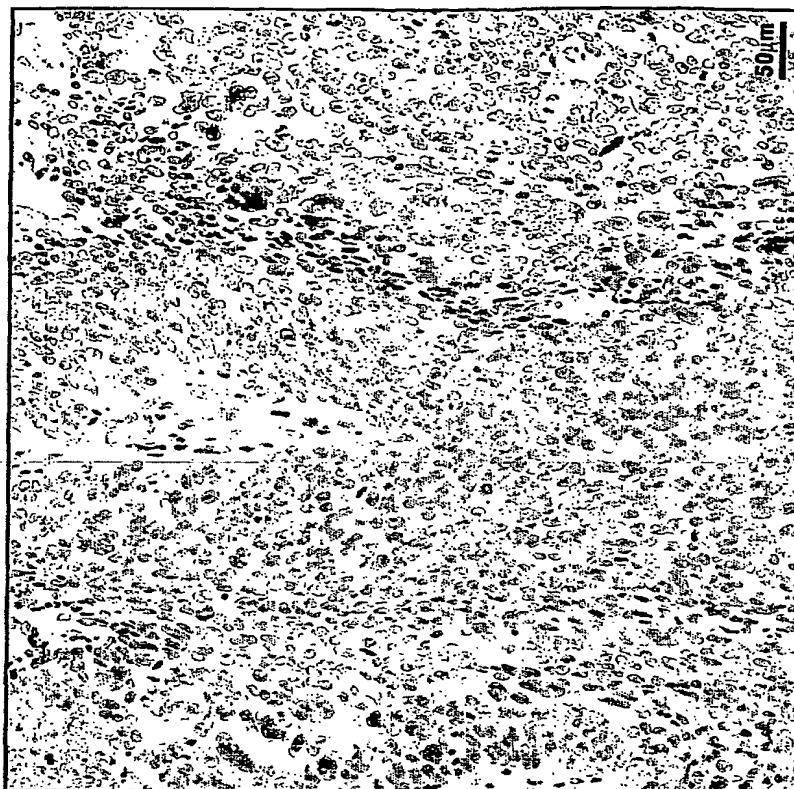
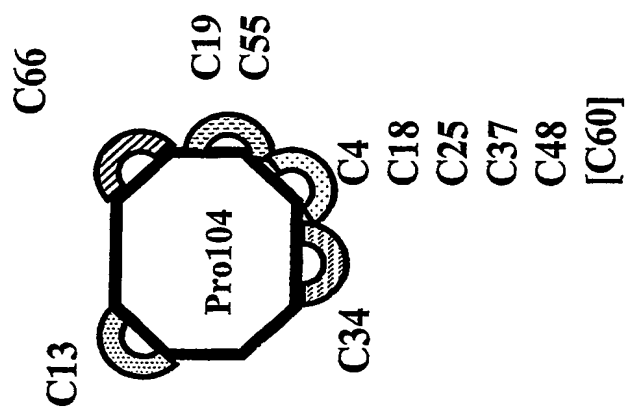


Fig. 13B Absorption
with Pro104 Antigen

Figure 14: Epitope Map of Pro104 MAbs



Pairs tested on Training Serum and Cell Line Panel:
C4/ C13; C48/ C13; C13/ C18; C19/ C48; C55/ C34; C66/ C18; C19/C25; C55/C25

FIGURE 15
Western Blot Showing Detection of Pro104 Protein in
mRNA+ Cell Lines and Ovarian Tumor Tissue (T) but not
Normal Adjacent Tissue (N)

Fig. 15A

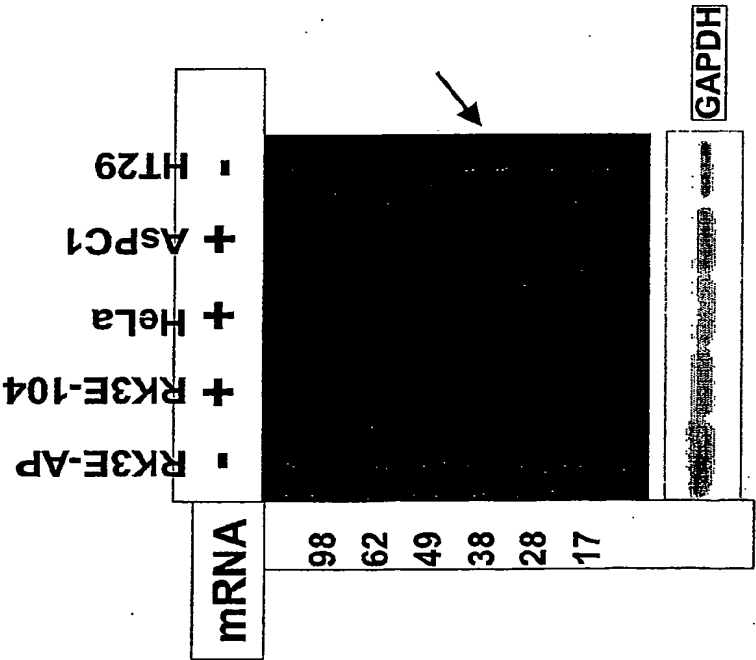
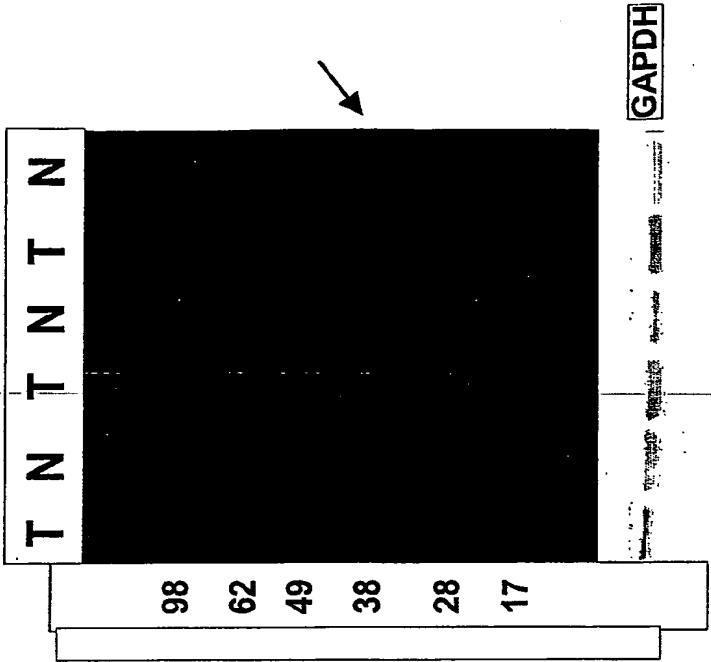


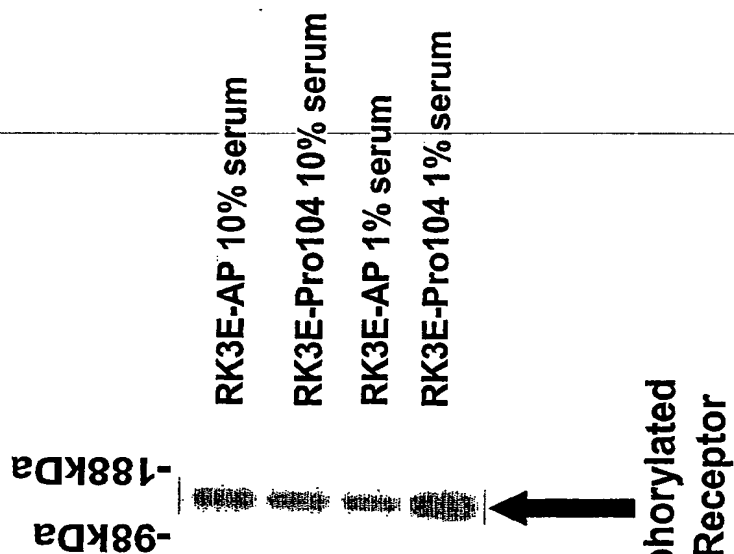
Fig. 15B



Arrow indicates location of Pro104 band

FIGURE 16

Overexpression of Pro104 Leads to Phosphorylation of EGF Receptor



Immunoblot With Antibody Against Phosphorylated EGF Receptor

FIGURE 17
Pro104 Protein is Glycosylated and GPI-Linked

Fig. 16A

Native Pro104 is de-glycosylated in
HeLa cell lines and ovarian tumors
when treated with PNGaseF

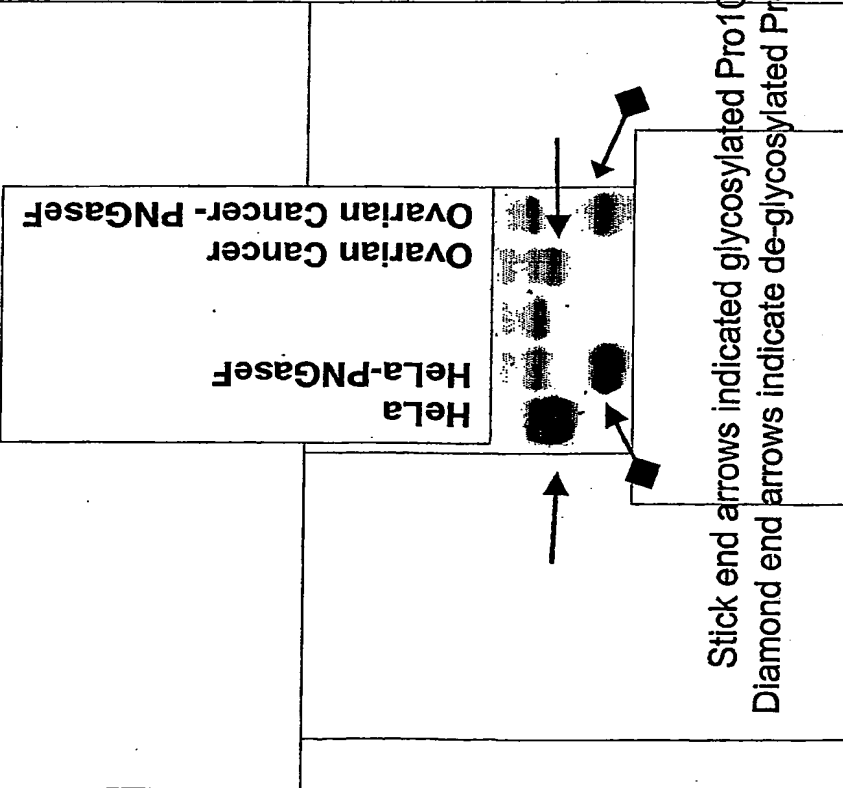


Fig. 16B

Native Pro104 shed into the media
when treated with PI-PLC

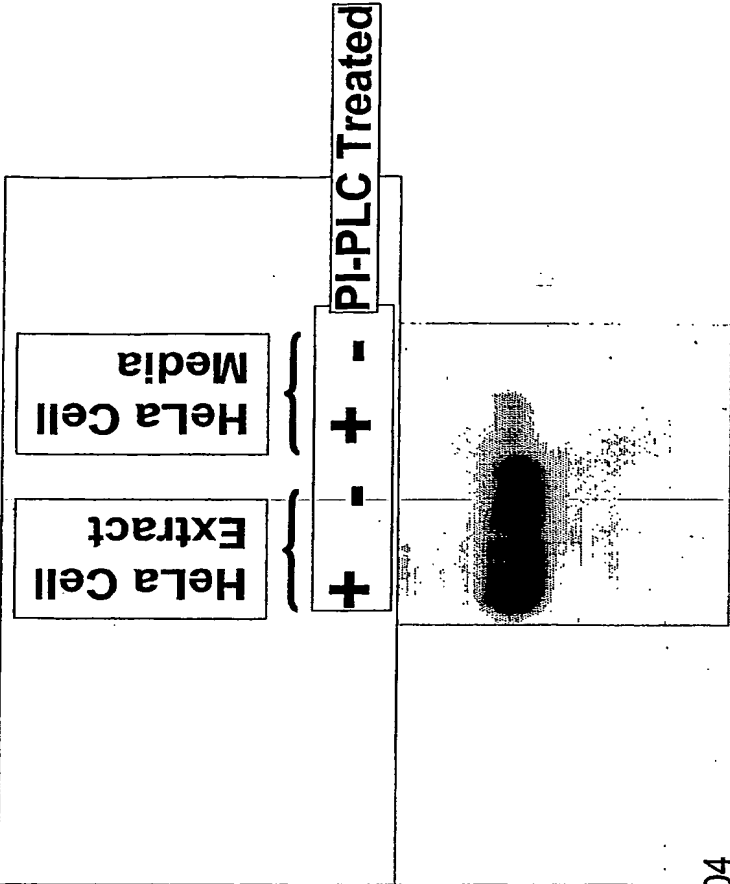


FIGURE 18
Surface Biotinylation of Native Pro104 in Cell Lines

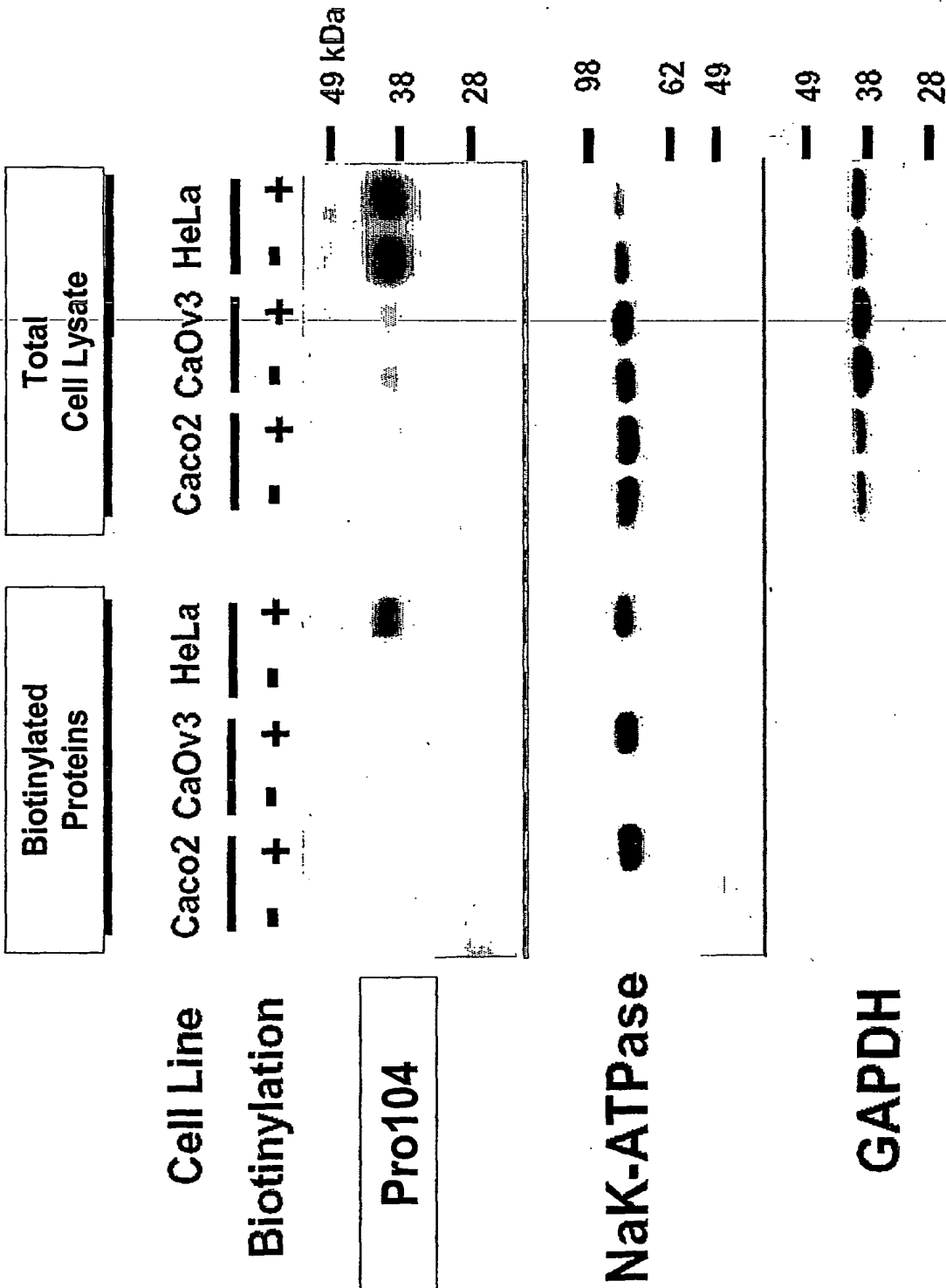
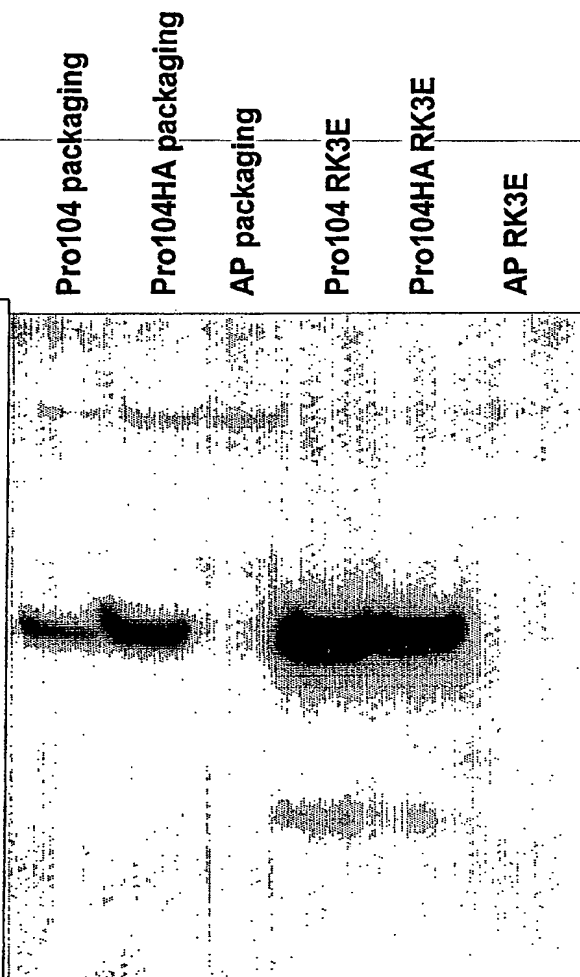


FIGURE 19

**Retroviral-Mediated Overexpression of
Pro104 Protein in RK3E Cells**

Pro104

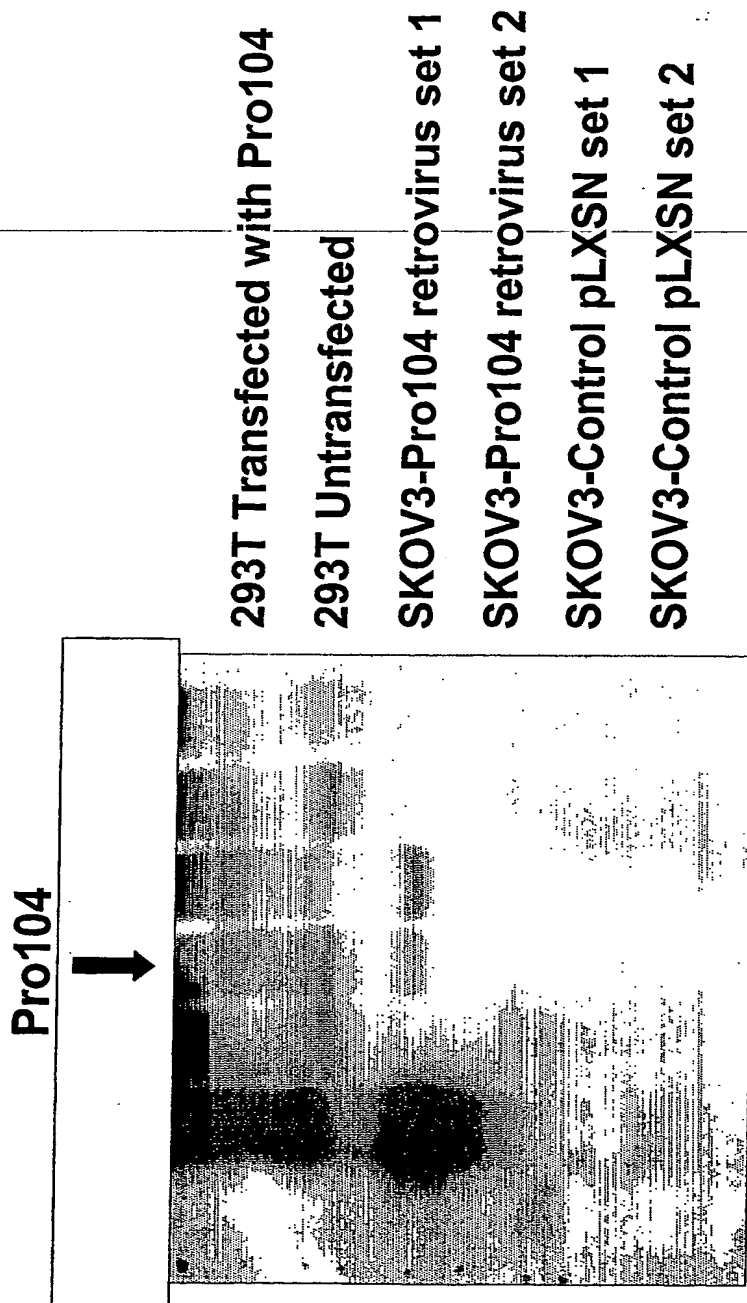


Blot Anti-Pro-104

Western Immunoblot demonstrating Pro104 protein expression in retroviral packaging cell lines and virus infected RK3E cells

FIGURE 20

Retroviral-Mediated Overexpression of Pro104 Protein in SKOV3 Cells

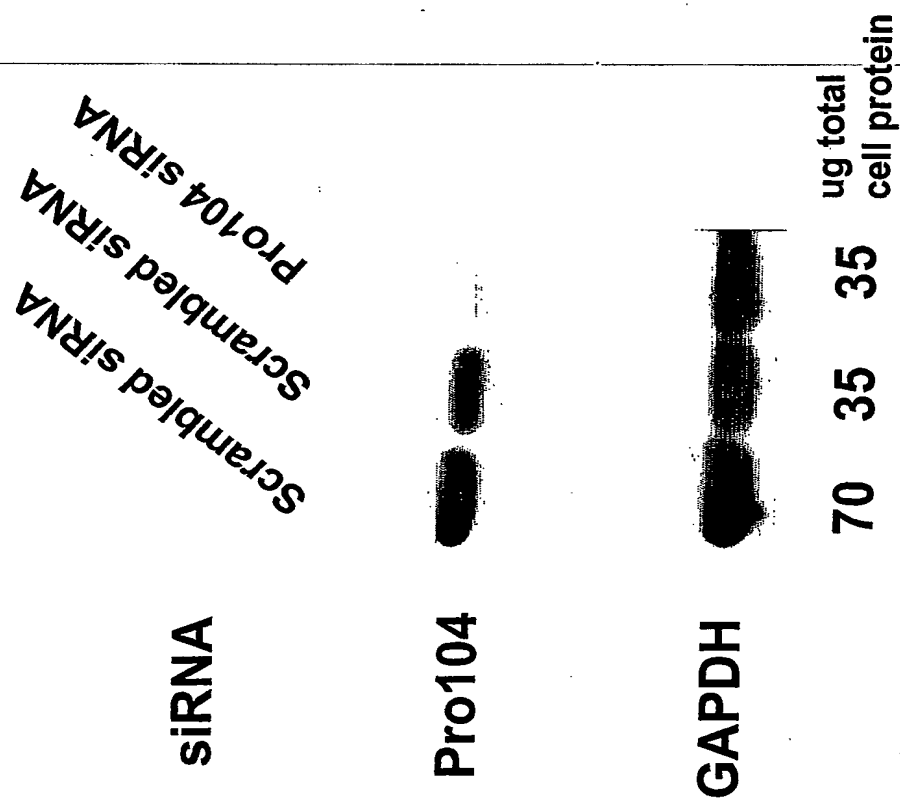


Blot Anti-Pro-104

Western Immunoblot demonstrating Pro104 protein expression in retroviral packaging cell lines and virus infected SKOV3 cells

FIGURE 21

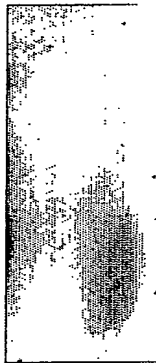
siRNA Mediates Specific Down-Regulation of Pro104 Protein in HeLa Cells



$\Delta CT = 1.3$ (knockdown 60%)

FIGURE 22
siRNA Mediates Down-Regulation of
Pro104 Protein in CaOV3 Cells

Scrambled siRNA
Pro104 siRNA



Pro104



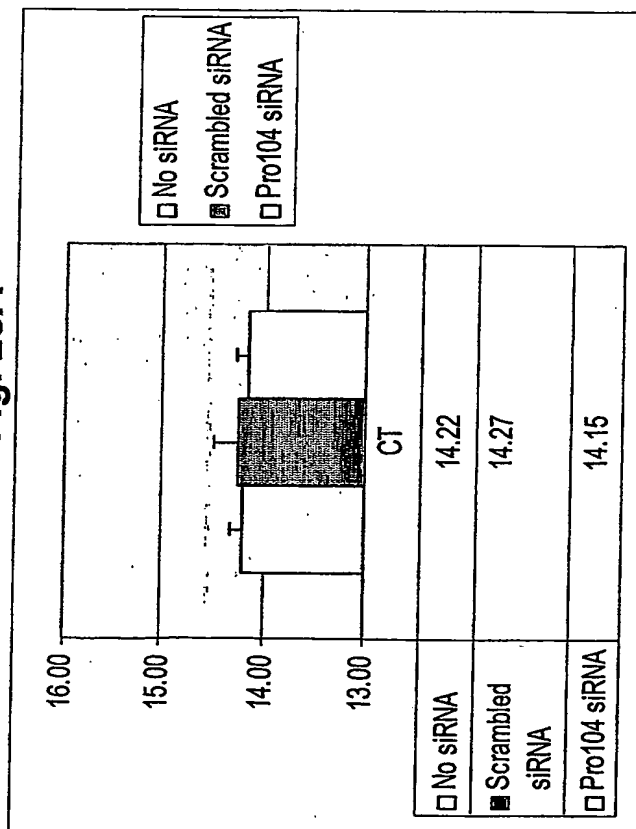
GAPDH

$\Delta CT = 1.2$ (knockdown 55%)

FIGURE 23

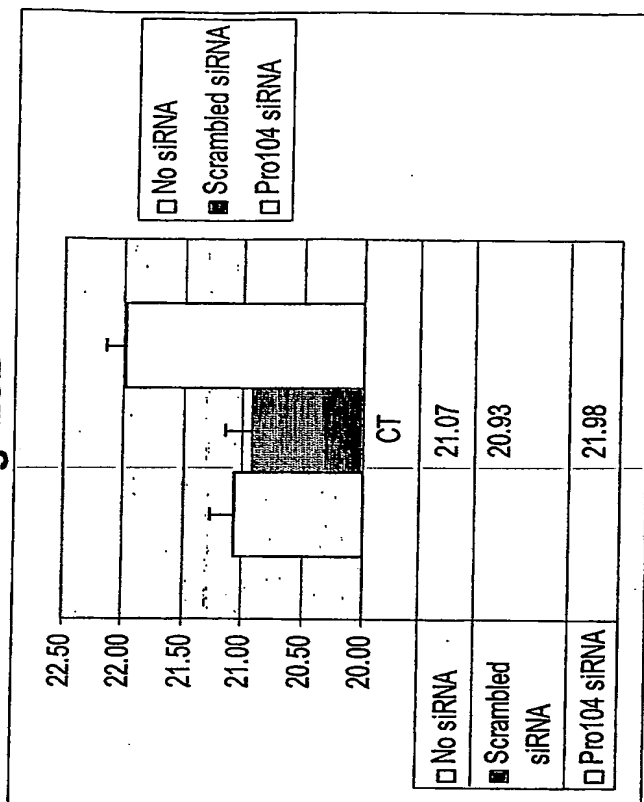
Pro104 siRNA Specifically Knockdown Pro104 mRNA in CaOV3 Cells

Fig. 23A



GAPDH Q-PCR Primers

Fig. 23B

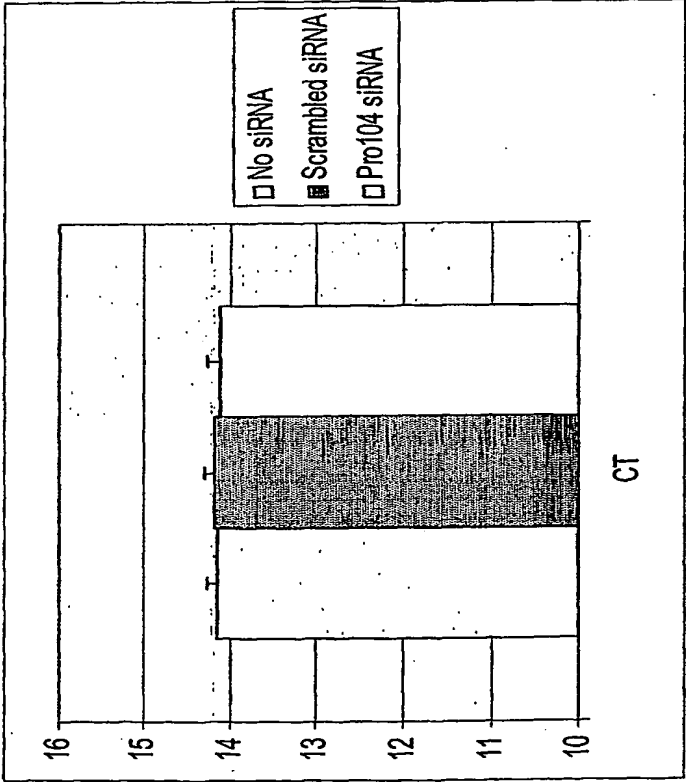


Pro104 Q-PCR Primers

$\Delta CT = 1$ for Pro104 (Knockdown 50%)

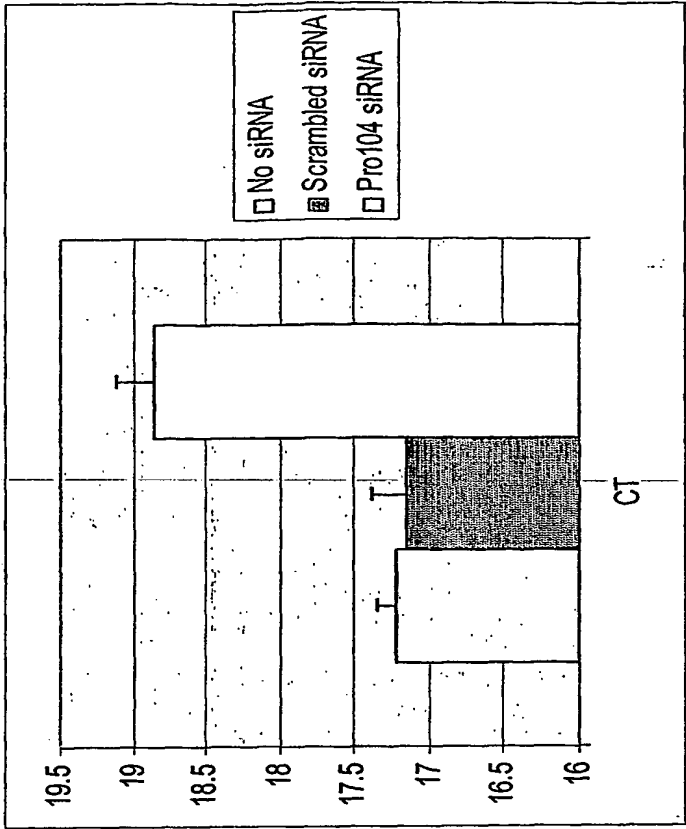
FIGURE 24
Pro104 siRNA Specifically Knockdown
Pro104 mRNA in HeLa Cells

Fig. 24A



GAPDH Q-PCR Primers

Fig. 24B

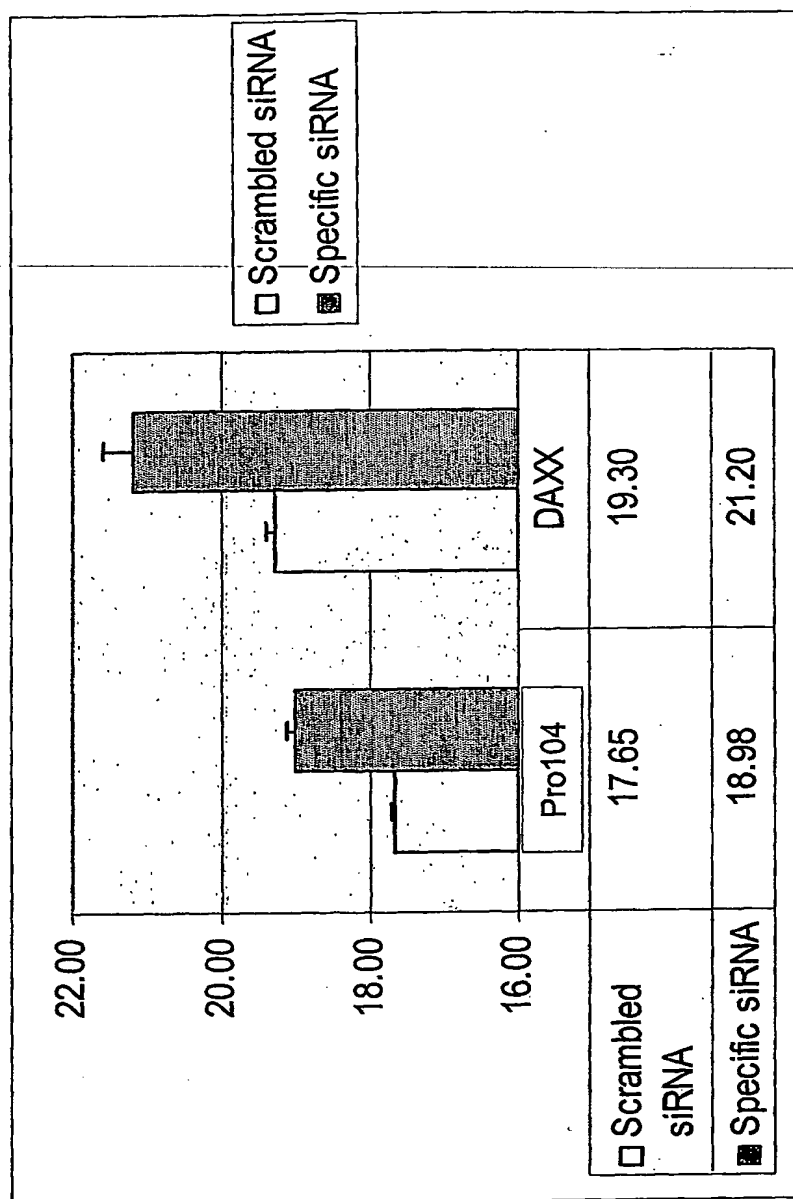


Pro104 Q-PCR Primers

$\Delta CT = 2$ for Pro104 (Knockdown 75%)

FIGURE 25

Pro104 siRNA Specifically Knockdown Pro104 mRNA in HeLa Cells, Compared to a Positive Control

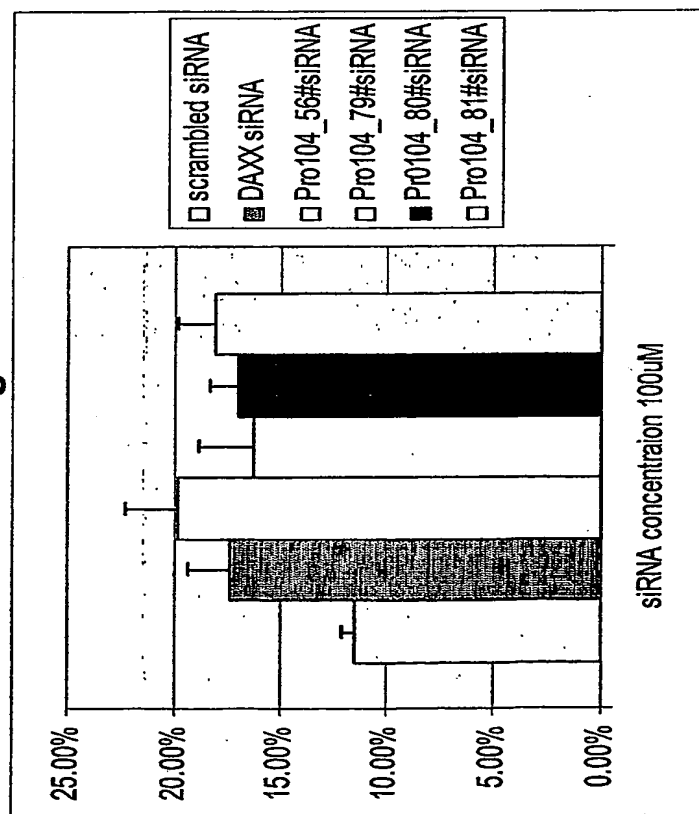


**QPCR with specific primers for Pro104 and DAXX
(positive control for apoptosis) demonstrates mRNA knockdown**

FIGURE 26

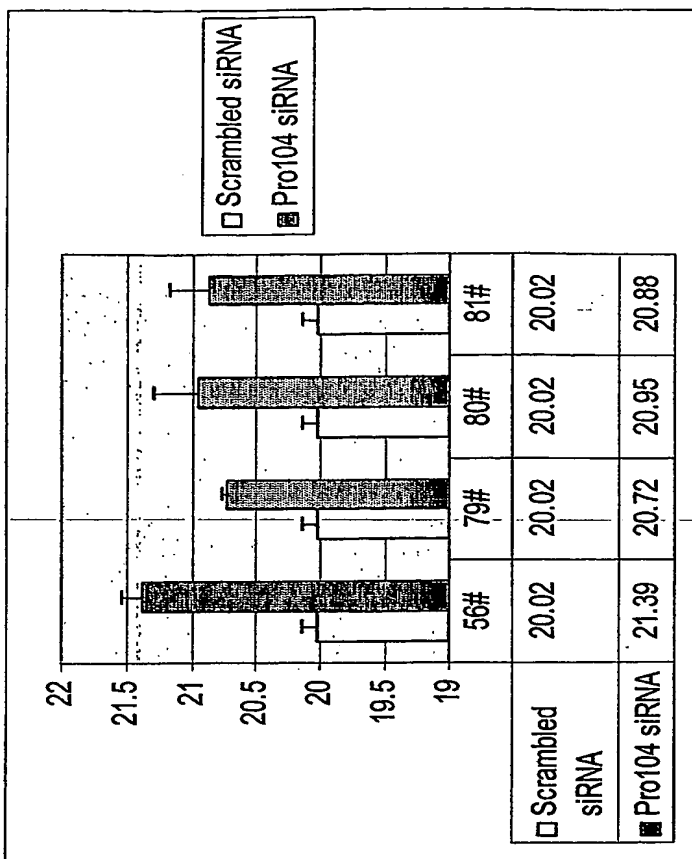
Different Pro104 siRNAs Induce Specific mRNA Knockdown and Apoptosis in HeLa Cells

Fig. 26A



Annexin V assay for apoptosis
(DAXX positive control)

Fig. 26B



QPCR demonstrates Pro104 mRNA
knockdown

FIGURE 27

Specific Knockdown of Pro104 mRNA in HeLa Cells Induces Cell Death

Fig. 27A

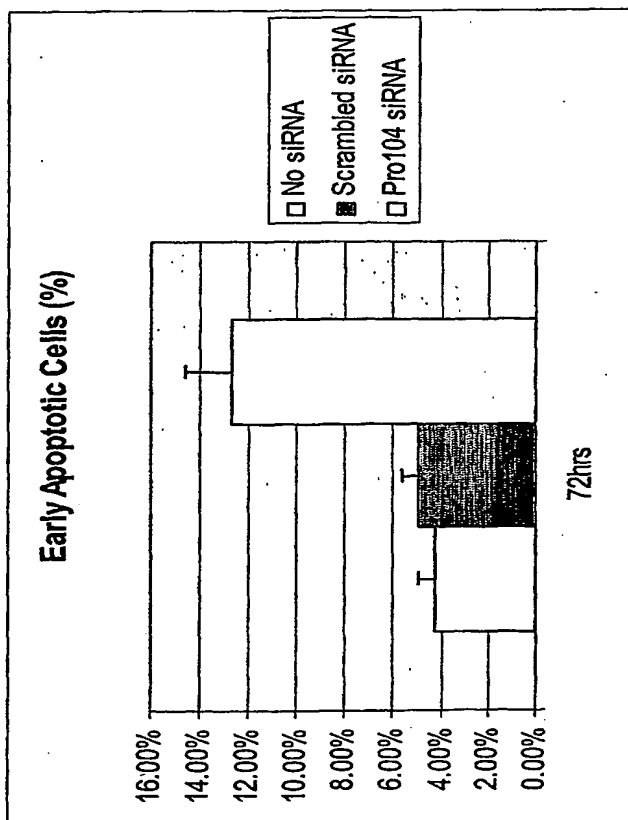
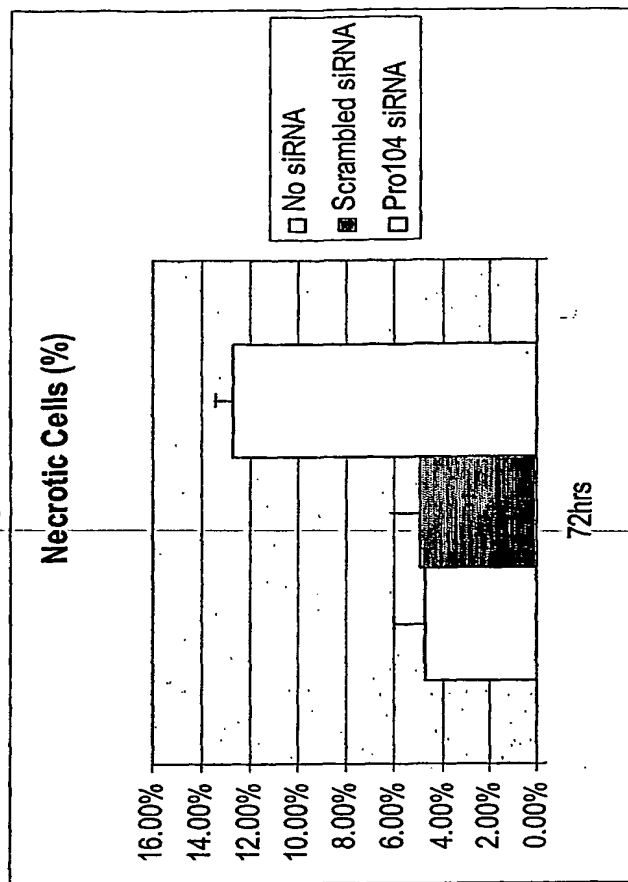


Fig. 27B



Annexin V Assay

FIGURE 28

Specific mRNA Knockdown by Pro104 siRNA Induces Apoptosis in HeLa Cells; Measured by Two Different Methods

Fig. 28A

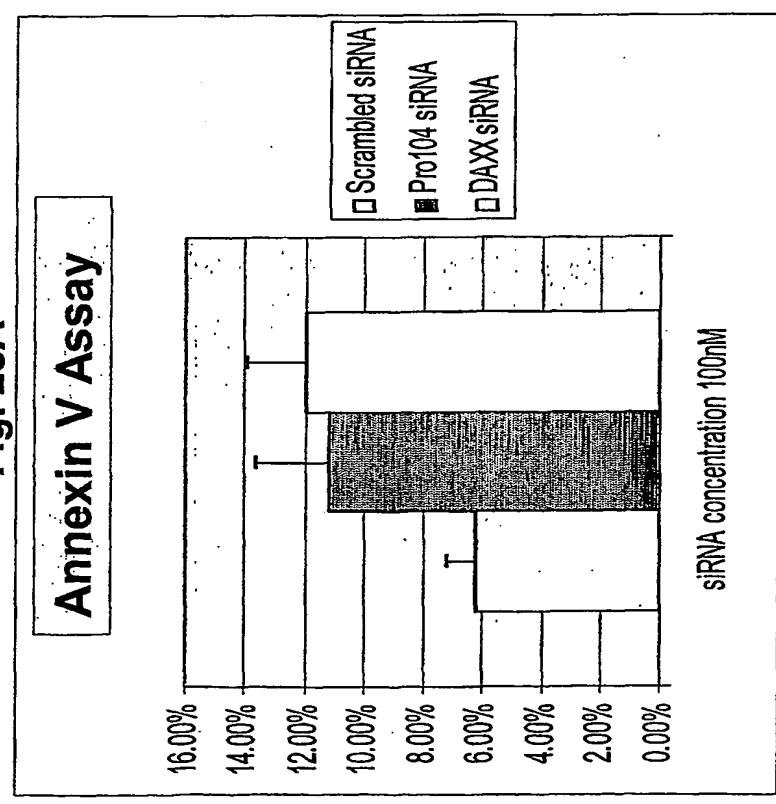
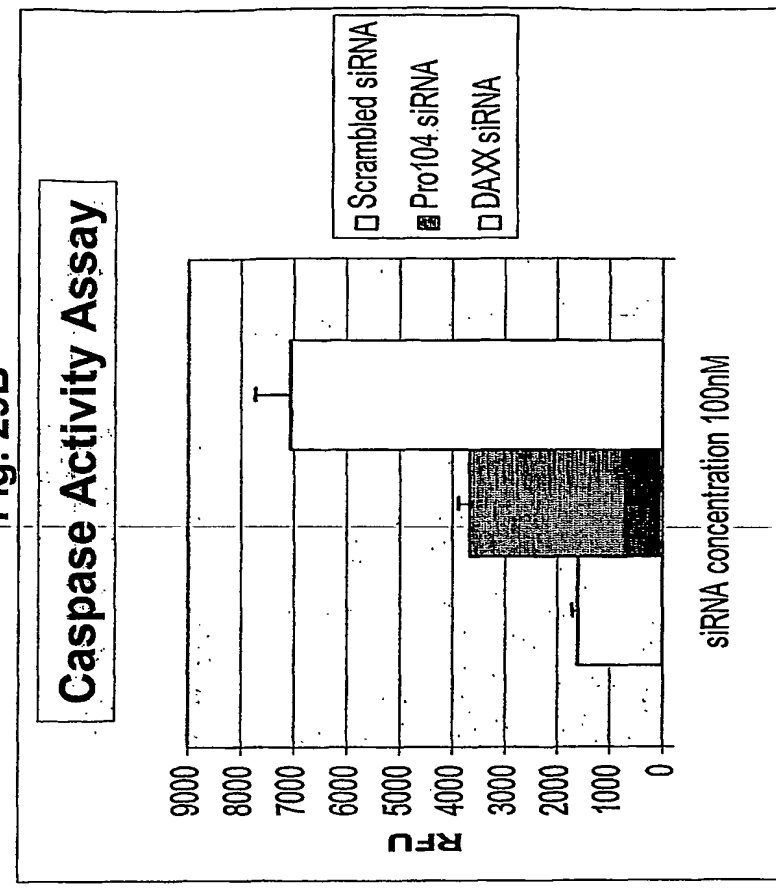


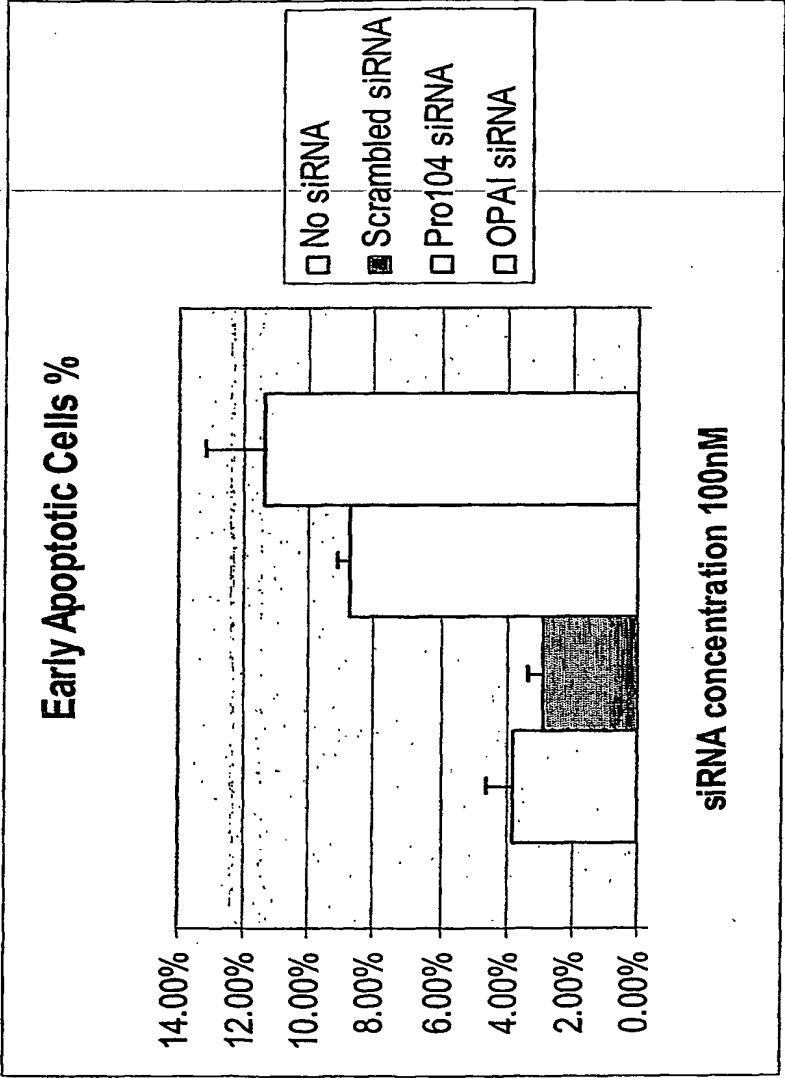
Fig. 29B



Apoptosis assays of cells treated with Pro104 siRNA

FIGURE 29

**Specific Knockdown of Pro104 mRNA
in CaOV3 Cells Induces Apoptosis**



OPAI: Positive Control

Annexin V Assay

FIGURE 30

Pro104 siRNA Has no Effect on Apoptosis in Cells Without Pro104 mRNA

Fig. 30A

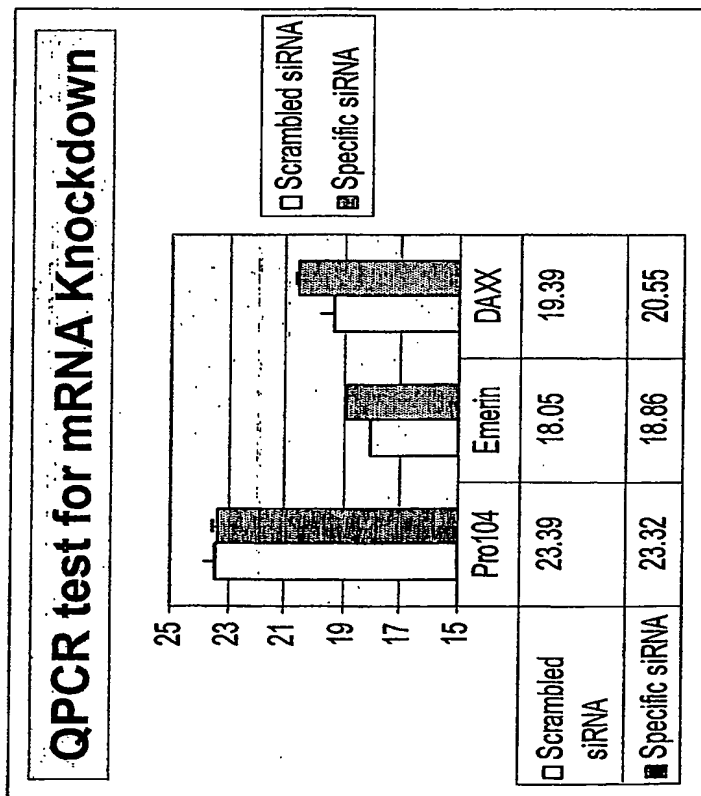
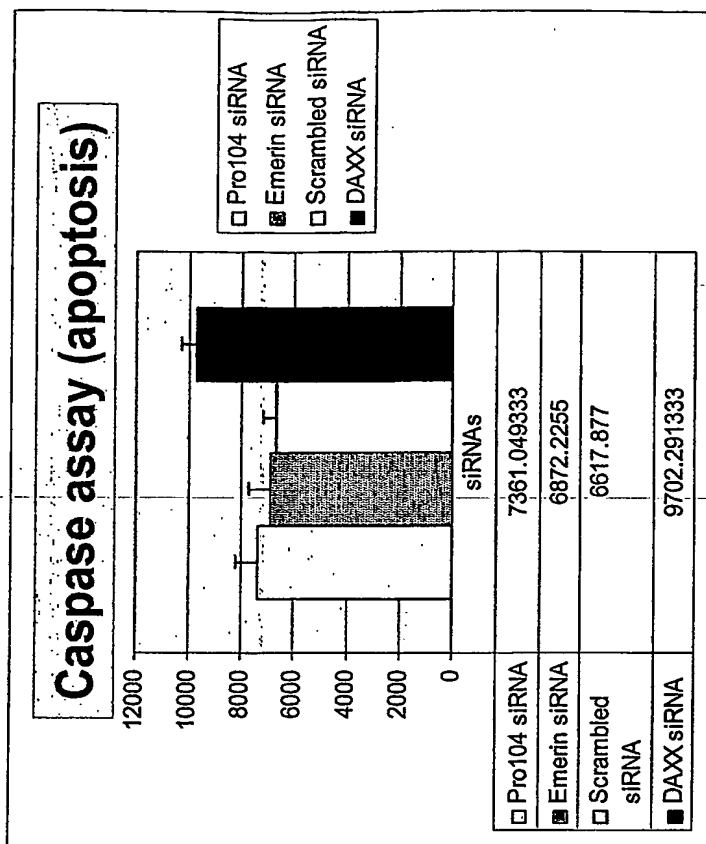


Fig. 30B



SKBR3 cells

Knockdown:

Pro104: none (no mRNA)

DAXX: 65% (positive control)

Emerin: 50% (mRNA +, non-essential)

FIGURE 31

Overexpression of Pro104 Induces Cell Growth in Soft Agar

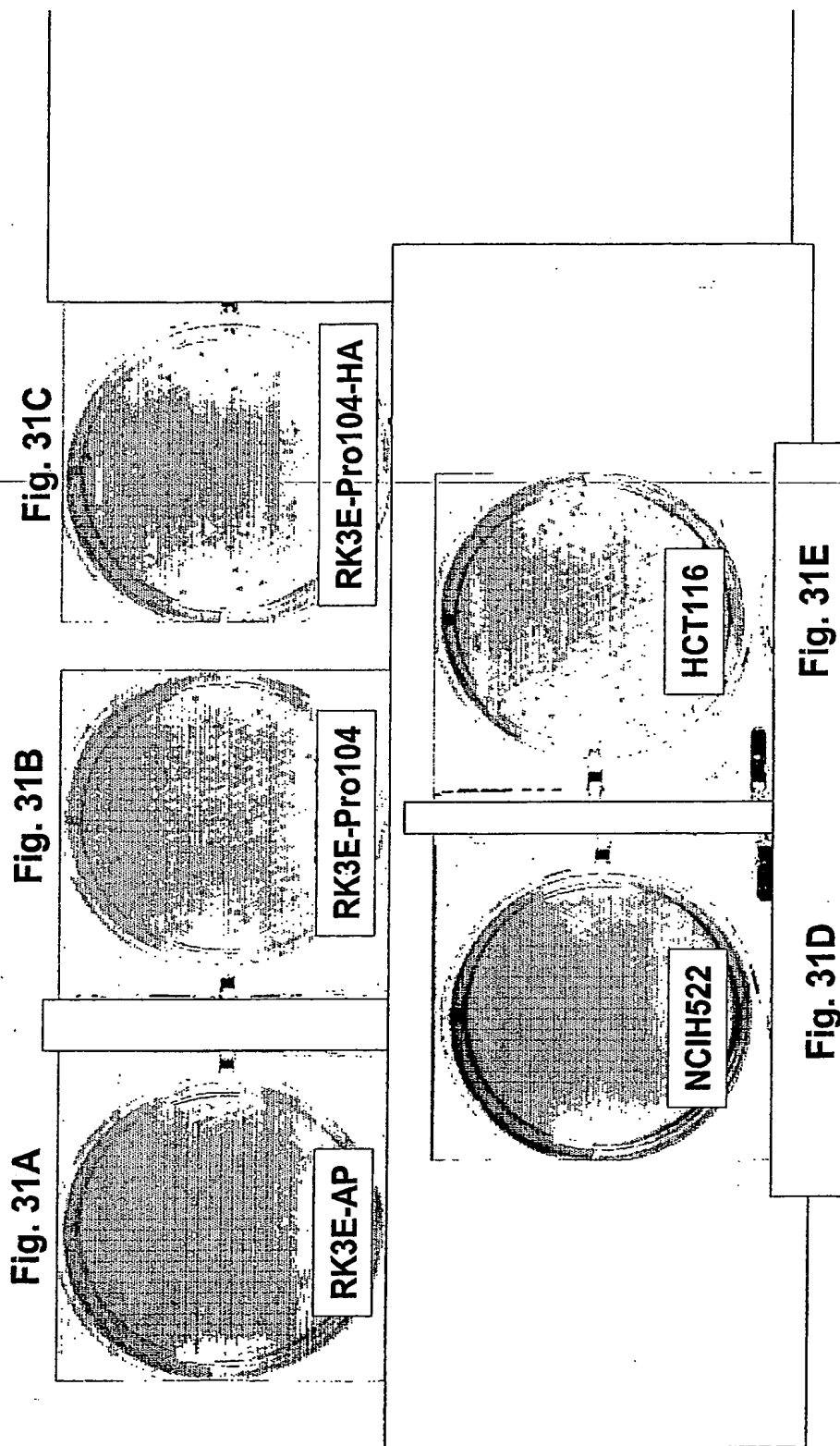


FIGURE 32
Pro104 Protease Activity is Required for Cell Growth

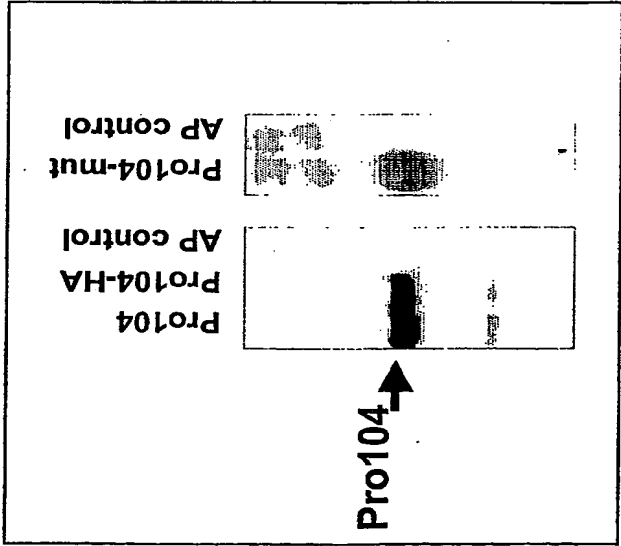


Fig. 32A

Fig. 32C

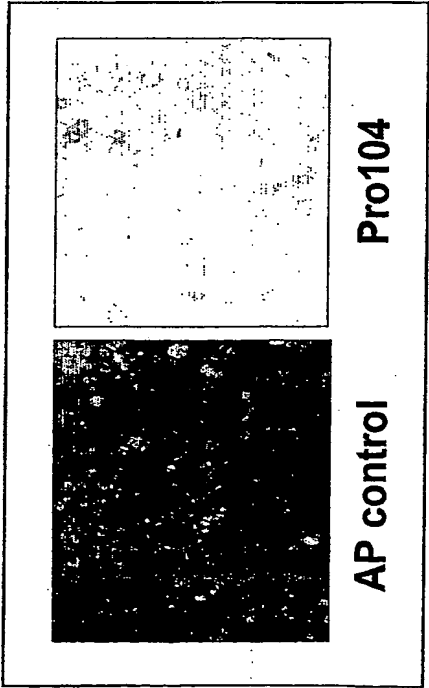
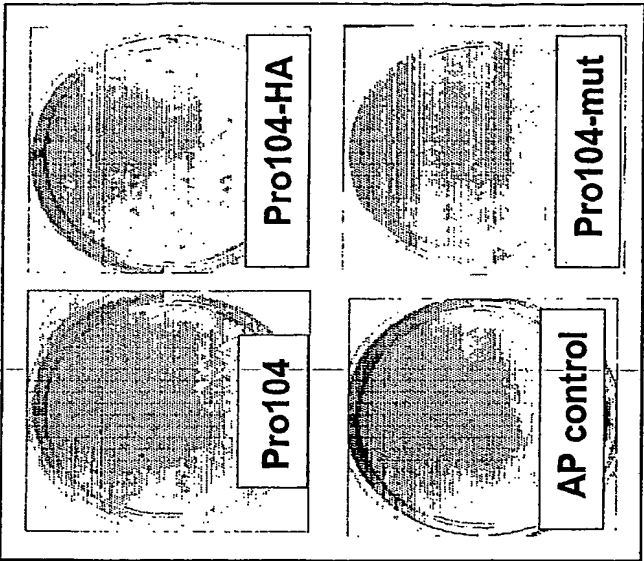


Fig. 32B

Cell Type	# Colonies per field
AP control	0
Pro104	60
Pro104-HA	68
Pro104-mut	0

Fig. 32D

RK3E cells with Pro104-mut lack Pro104 protease activity

FIGURE 33

Knockdown of Pro104 mRNA by siRNA

Inhibits Growth of HeLa Cells in Soft Agar

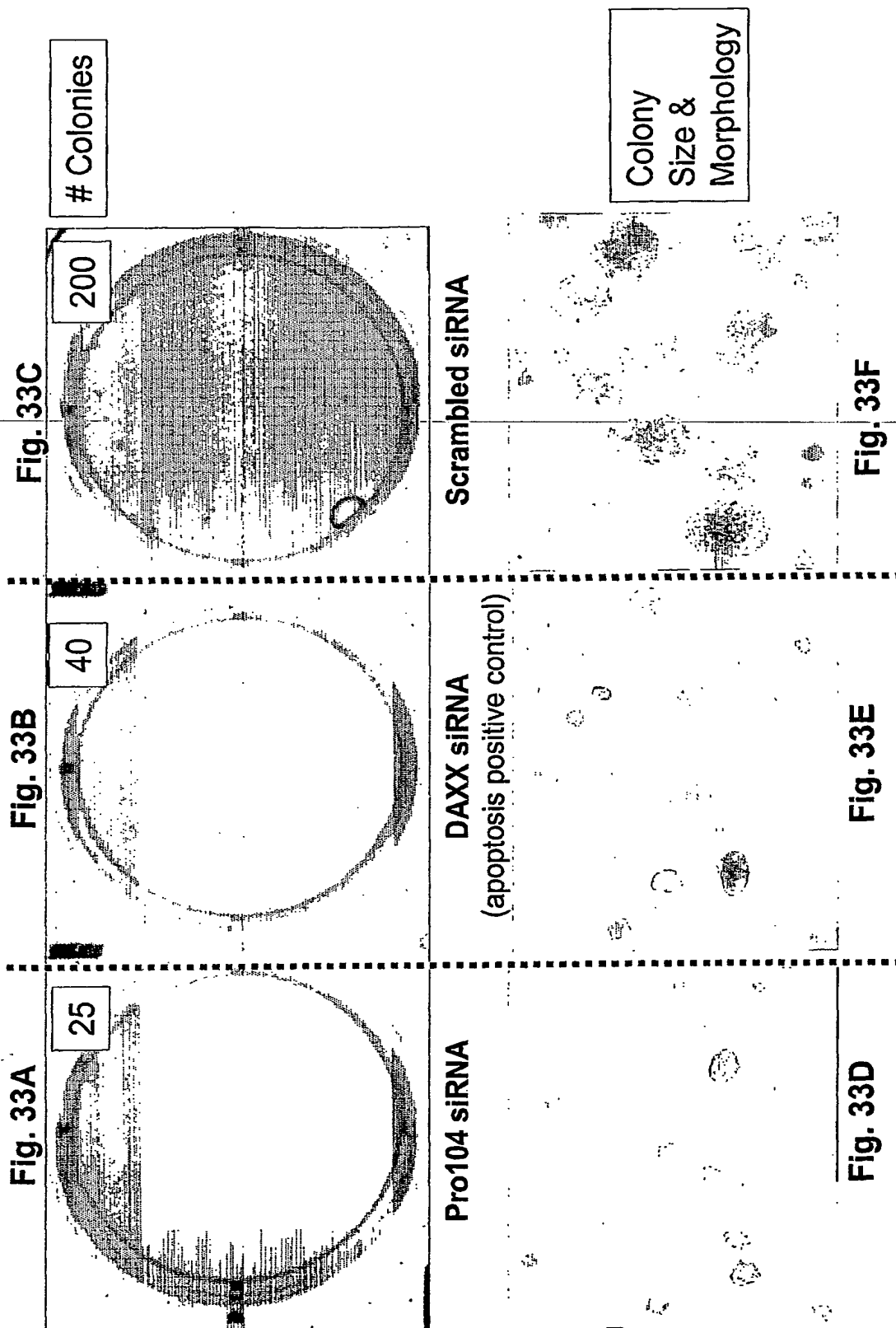
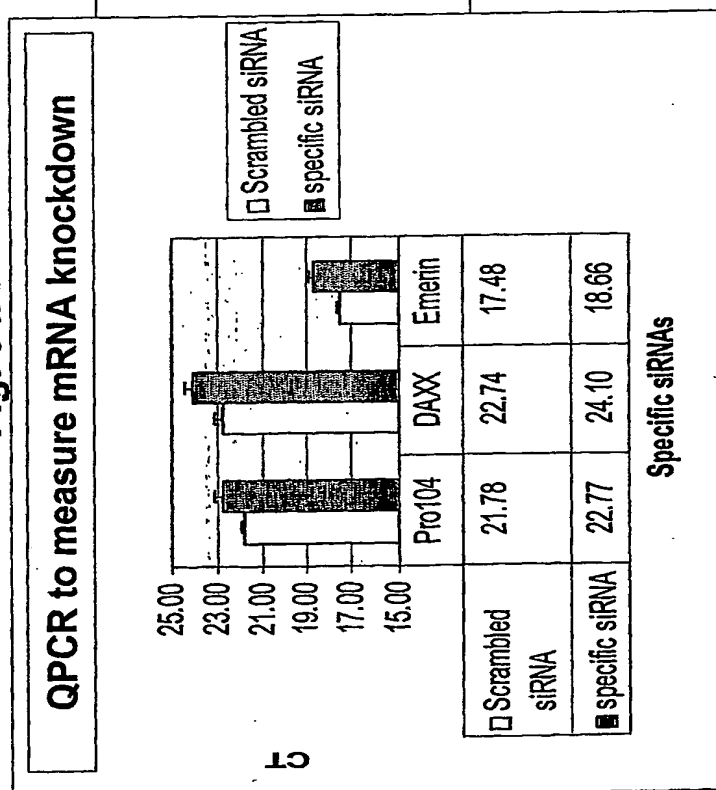


FIGURE 34

Knockdown of Pro104 mRNA by siRNA Inhibits Growth of HeLa Cells in Soft Agar

Fig. 34A



Knockdown:
Pro104: 50%
DAXX: 65%
Emerin: 50%

Fig. 34B

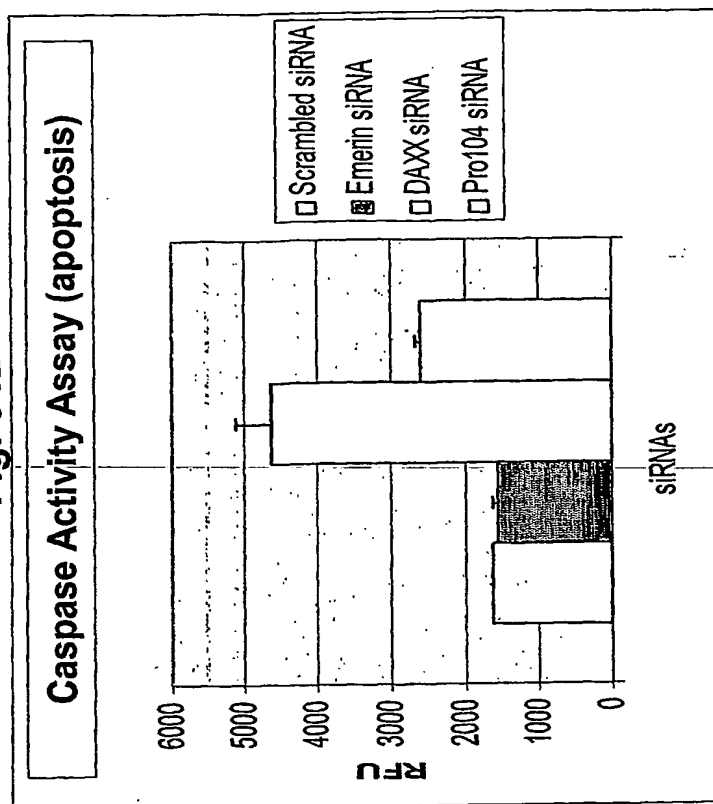


FIGURE 35

Increased Growth of Human Tumor Cells Over-Expressing Pro104

Fig. 35A

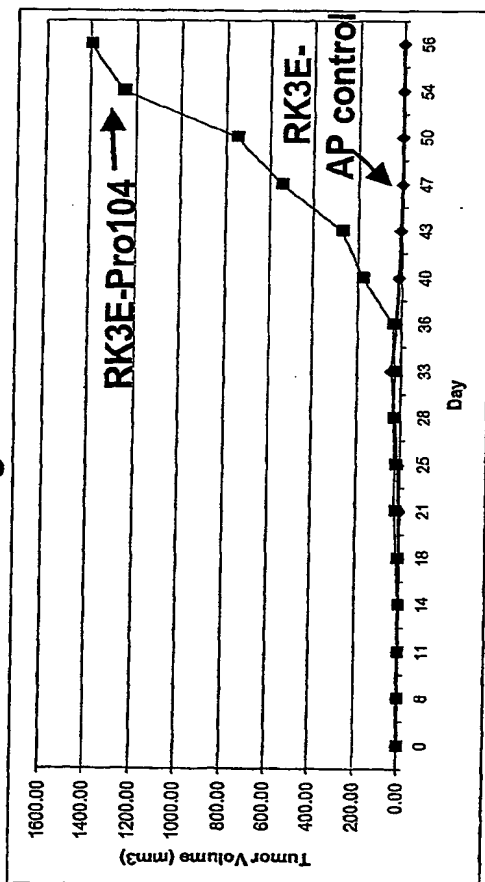


Fig. 35B

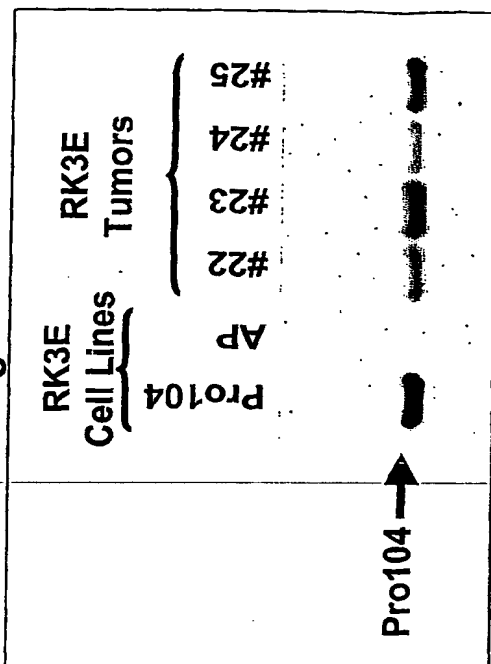


Fig. 35C

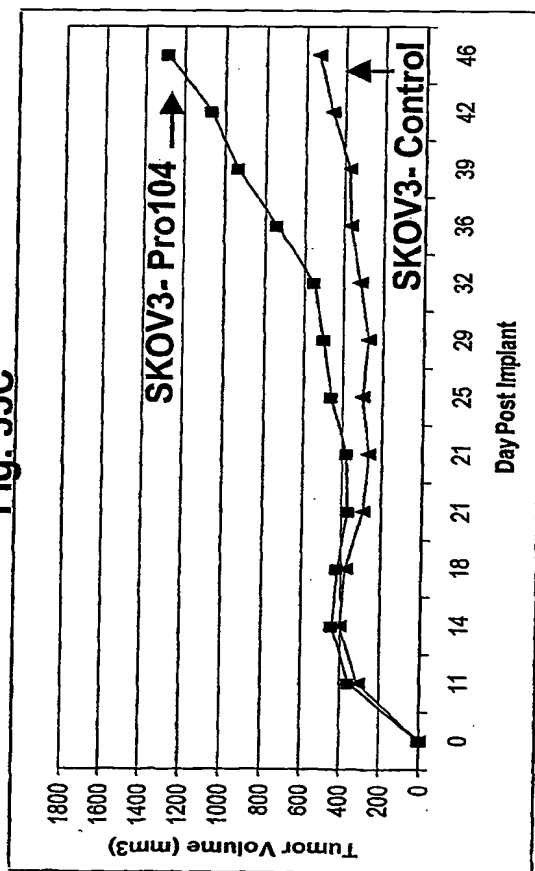
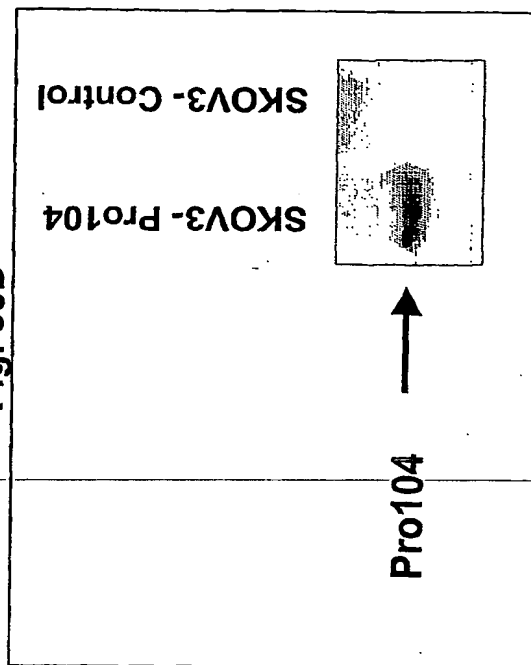


Fig. 35D



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